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Morphology of Acute Promyelocytic Leukemia With Cytogenetic or Molecular Evidence for the Diagnosis: Characterization of Additional Microgranular Variants

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Early diagnosis of t(15;17) acute promyelocytic leukemia (APL) is essential because of the associated disseminated intravascular coagulation and the unique response of the disease to all-trans retinoic acid (ATRA) therapy. Early diagnosis depends primarily on morphological recognition. The French-American-British (FAB) classification, however, does not describe all morphological variations that occur in APL. In 25 cases with evidence of APL confirmed by cytogenetic and/or molecular analysis, we found a heterogeneous morphological group. The most common form of APL was heterogeneous and consisted of various combinations of cells in which hypergranular cells and some cells with multiple Auer rods were obvious. In some cases, one cell predominated. This led to the description of five subcategories. These included the classical FAB M3 with hypergranular cells and multiple Auer rods; the FAB variant with hypogranular bilobed cells; the basophilic cell type of McKenna et al. [Br. J. Haematol 50:201, 1982]; and two additional subtypes, one consisting of differentiated promyelocytes and a few blast cells (M2-like), and the other consisting largely of blast cells and a few early promyelocytes (M1-like). Immunophenotyping revealed a pattern of CD33 and/or CD13 positivity, and CD14 and HLA-DR negativity in 96% of cases. CD2 was positive in the FAB variant and in the subtype with basophilic cells, but negative with other subtypes. Three out of five cases with basophilic cell predominance [McKenna et al.: Br J Haematol 50:201, 1982], and one out of two M2-like cases, responded to ATRA therapy. Awareness of the heterogeneity and the atypical morphologic subtypes found in t(15;17) APL will contribute to improved recognition and early institution of ATRA therapy. Am. J. Hematol. 56:131–142, 1997. © 1997 Wiley-Liss, Inc.

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INTRODUCTION

The 15;17 translocation [t(15;17)(q22;q21)] is associated with the pathogenesis of acute promyelocytic leukemia (APL) in both the classical and variant subtypes [1,2]. Cytogenetically detectable rearrangements are found in 70–80% of cases [3,4], but combined cytogenetic and molecular findings suggest that t(15;17) is found in more than 90% of APL cases [3,5]. The reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of promyelocytic/retinoic acid receptor α leukemia (PML/RARα) transcripts is the most sensitive and specific method to identify AML patients with t(15;17) [6], but requires experience with RT-PCR technology and RNA manipulation [3,7,8]. The 15;17 translocation has not been observed in other types of neoplasia [3,9]. The breakpoints involve the retinoic acid receptor α gene (RARα) on chromosome 17 and the PML gene on chromosome 15 [10,11]. The chimeric gene PML/RARα is

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expressed on 100% of t(15;17)-positive APL cases and RARα/PML on about 70% [12]. An 11;17 translocation has been found infrequently in APL [5,13,14]. Rare cases of t(5;17) APL [15,16] have been reported. In both, the reciprocal chromosomal translocation also involved the RARα gene [15,17]. The RARα gene rearrangement is considered a molecular marker of APL [2,11,15], consistent with a model for the molecular control of myeloid maturation whereby the formation of functional RXR-RARα heterodimers is required for differentiation beyond the promyelocyte stage [18–20].

Prompt recognition of APL is important because of the associated disseminated intravascular coagulation (DIC) [3,21] which is often aggravated or precipitated by chemotherapy [22–24], with resultant hemorrhage and death. Early diagnosis is also important because all-transretinoic acid (ATRA) therapy is uniquely effective in t(15;17) APL [3,8,25]. ATRA therapy induces the malignant promyelocytes to differentiate [3,4,23], possibly alleviates the hemorrhagic diathesis [3], and improves the survival of APL patients [24,26,27]. Although cytogenetic and molecular studies allow a precise diagnosis of APL [3], the peripheral blood and bone-marrow morphology are often the first indication that prompts special investigation.

The French-American-British (FAB) classification [28,29] is used by most morphologists to diagnose APL; however, it does not describe all the morphologic variations that occur in APL. In 1982, McKenna et al. [21] described 3 among 39 APL cases where the predominant cell was small with a hyperbasophilic cytoplasm, cytoplasmic projections, and fine or no visible granules, resembling on occasion a micromegakaryocyte [30]. They included these cases under the heading ''microgranular'' APL [21].

We have reexamined the bone-marrow aspirates of 25 acute myeloid leukemia (AML) cases, referred to the Hamilton Regional Cell Typing Laboratory, that had shown objective evidence of APL by cytogenetic or molecular genetic analysis. In analyzing these cases, we have found five major morphologic subtypes of APL, including the two described by the FAB Co-operative Group [1,28] and the microgranular type described by McKenna et al. [21]. In addition, two other subtypes were noted, one consisting mainly of normal-appearing, differentiated promyelocytes, and the other largely of myeloblasts [31]. In this paper, we describe the morphology of the 25 cases together with their cytogenetic, molecular, and immunophenotypic findings.

MATERIALS AND METHODS

Patient Population

Five hundred and eighty-nine patients were diagnosed as de novo AML by combined morphologic-cytochemical examination and immunophenotyping between January 1, 1984–October 16, 1996. Forty-one cases (7%) were APL, of which 24 cases demonstrated hypergranular morphology (FAB M3, 59%). The other 17 cases were variant forms (41%). Of the 41 cases, 25 had samples taken for karyotypic examination, and/or fluorescence in situ hybridization (FISH) or Southern blot analysis of the PML and RARα loci or, more recently, for PML/RARα transcripts by polymerase chain reaction (PCR). Clinical and laboratory data were available on all patients. Laboratory evidence of DIC was based on a fibrinogen level of <1.0 g/l without evidence of liver disease, and a positive protamine sulfate test or D-dimer test. Of the 25 patients, 20 were managed at Hamilton hospitals and 5 at other institutions. All clinical specimens were obtained after informed consent from the patient.

Cytogenetic and FISH Analysis

Bone-marrow aspirates were collected into sodium heparin at diagnosis for cytogenetic analysis and were either directly analyzed, or analyzed after culturing in RPMI-1640 for 24 hr at 37°C and 5% CO_2 in a humidified atmosphere. Routine harvesting methods were used to prepare slides for G-banding, digital imaging, and computer-assisted karyotyping (PSI Instrument, League City, TX).

For FISH analysis, metaphase and interphase cells were subjected to two-color fluorescence analysis after in situ hybridization with a digoxigenin-labelled 15;17 probe set, applied according to the manufacturer's recommendations (P5119-D/B, Oncor, Gaithersburg, MD). Probe sequences labelled with rhodamine (17q21 locus) or fluorescein (15q22 locus) fluorescence were visualized in a Zeiss Axioplan microscope (Carl Zeiss, Canada, Toronto, Ontario) fitted with a dual band-pass filter.

Southern Blot Analysis [32]

High-molecular weight DNA was digested with the restriction enzymes *Eco*RI, *Hind*III, *Bgl*I, or *Bam*HI, size-fractionated by 0.7% agarose gel electrophoresis, and transferred to a nylon membrane (Hybond, Amersham, Oakville, Canada). Membranes were hybridized with probes labelled with ^{32}P by random priming [33]. Autoradiography was undertaken with intensifying screens at −70°C. Southern blot was performed on 12 of the 25 cases and also on 12 cases of non-M3 AML.

DNA Probes

The probes used to study the RARα locus were H18, a 0.6-kb *Hind*III-*Eco*RI fragment, and HB, a 1.7-kb *Hinc*II-*Bam*HI fragment, both derived from RARα genomic λ phages. To study the PML (Myl) locus, we used RH15, a 1.3-kb *Sma*I-*Hinc*II fragment derived from Myl genomic λ phages. All three were kindly provided by Dr. A. Biondi (Milan, Italy) [34].

RT-PCR

RNA was extracted from bone-marrow mononuclear cells, using the TRIzol reagent (Life Science Technolo-

gies, Inc., Gaithersburg, MD) according to the supplier's directions. The primers used for reverse transcriptase and PCR amplification to distinguish between these short, long, and variable forms were as described by Gallagher et al. [35]. RT-PCR, having only become available to us in the past year, was performed on 3 recent patients on admission and on 1 patients on follow-up.

Morphologic Examination

Wright-Giemsa (WG)-stained bone-marrow and peripheral blood preparations of the 25 cases with cytogenetic or molecular genetic evidence of APL were examined and subtyped by two pairs of morphologists (P.B.N. and P.S., and J.A.M. and W.P.). Each pair examined the peripheral blood and bone marrow jointly and reached a diagnosis. Any case where there was disagreement was reexamined by the senior members of the pair (P.B.N. and J.A.M.), and a consensus diagnosis was made. All subtyping was performed without knowledge of the clinical or immunophenotypic data. Scanning of smears for multiple Auer rods was performed by one investigator (W.P.). One pair (P.B.N. and P.S.) performed a differential of 300 cells on all bone marrows and of 100 cells on the peripheral blood specimens. The same pair also examined the cytochemical preparations, which included stains for myeloperoxidase (MPO) [36], Sudan black B (SBB) [37], chloracetate esterase (CAE) [38], and nonspecific esterase (NSE) [38]. Staining with toluidine blue was not performed.

Immunophenotyping

A panel of 13 monoclonal antibodies was used to establish the antigen profile of 24 APL cases tested using flow cytometry [39] (one case not tested). These included CD33 (My9), CD13 (My7), CD14 (My4), CD2 (T11), CD19 (B4), HLA-DR, and CD11b (MO1) from Coulter Immunology, Miami, FL; CD15 (Leu M1), CD5 (Leu1), and CD11c (Leu M5) from Becton Dickinson, San Jose, CA; and CD34 and CD117 from Immunotech, Westbrook, ME. During the same period, 524 cases of non-FAB M3 AML subtypes were immunologically typed with the same antibodies, and the profiles were compared with those obtained in the 24 FAB M3 cases. A positive reaction was defined as 20% of leukemic cells more fluorescent than the control [40].

RESULTS

Genetic Analysis

Cytogenetic analysis. Chromosome preparations were adequate for the analysis of 24 of the 25 cases. Metaphases adequate for karyotyping were not obtained in one case (case 19). The 15;17 translocation was found in 19 cases (79%). Additional karyotype abnormalities were found in 6 cases (cases 2, 3, 11–13, and 23) (Table I).

TABLE I. APL: Results of Cytogenetic and Molecular Genetic Analysis*

Case no.	Age/sex	Karyotype	FISH	Southern blot	RT-PCR
1	28/F	t(15;17)		ND	
2	40/F	t(15;17)[a]		ND	
3	47/M	t(15;17)[a]		ND	
4	70/F	t(15;17)		ND	
5	6/F	t(15;17)		RARα/PML G/R	
6	43/F	46, XX	+	PML G/R	+
7	35/M	t(15;17)		RARα G/R	
8	47/F	t(15;17)		ND	
9	57/M	t(15;17)	+	RARα/PML G/R	+
10	76/F	46, XX	+	ND	+
11	42/M	t(15;17)[a]		ND	
12	35/F	t(15;17)[a]	+	RARα G/R	
13	68/M	t(15;17)[a]			+
14	66/M	t(15;17)		ND	
15	64/F	t(15;17)		ND	
16	82/F	t(15;17)	+	PML G/R	
17	32/M	t(15;17)		RARα G/R	
18	58/F	t(15;17)		ND	
19	65/M	ND		RARα G/R	
20	17/F	t(15;17)		ND	
21	45/M	t(15;17)		RARα/PML G/R	
22	53/F	46, XX	+	RARα/PML G/R	
23	54/M	t(15;17)[a]		ND	
24	60/F	46, XX		RARα G/R	
25	73/F	46, XX		RARα/PML G/R	

*+, positive; G/R, germline/rearranged; ND, not done.
[a]Additional genetic abnormality.

FISH. FISH was positive for t(15;17) in 6 cases examined (cases 6, 9, 10, 12, 16, and 22).

Southern blot analysis. Rearrangement of either the RARα or PML locus was observed in all 12 patients studied. In 7 of these, t(15;17) was also observed by cytogenetic analysis [9]. Using the three probes, two derived from chromosome 17 and one from chromosome 15 and two restriction enzymes (*Eco*RI and *Hind*III), rearrangements of PML or RARα loci were detected in all 12 cases tested [34]. In 4 patients (cases 17, 19, 24, and 25), no PML rearrangement was detected after digestion with either *Eco*RI or *Hind*III. In one case (case 17), t(15;17) was detected by cytogenetic analysis. In another (case 25), digestion with *Bgl*I or *Bam*HI demonstrated a rearranged PML band.

RT-PCR. RT-PCR was performed in 3 recent cases (cases 9, 10, and 13). All cases were positive and all showed evidence of the short form of the mRNA transcript (breakpoint in intron 3 of PML). A further case (case 6), on follow-up analysis 11 months after diagnosis, was positive, showing the long form of the mRNA transcript (breakpoint in intron 6 of PML).

In summary, of the 25 cases, evidence for APL was present in all cases by cytogenetic or molecular genetic analysis. In 19 patients, t(15;17) was shown by conventional karyotyping. FISH was positive in 3 patients (cases 6, 10, and 22) with a normal karyotype. Of the 3 remain-

TABLE II. Bone–Marrow Differential (%) on 25 APL Cases*

Case no.	Hypergranular cells	Cells with multiple Auer rods differential/scan		Fine dusting granules	Bilobed variant cells	Basophilic cells without granules	Basophilic cells with granules	Basophilic cells with Auer rods	Promyelocytes	Blast cells	Myelocytes to neutrophils	Lymphocytes	APL subtype[a]
1	31	0	−	<1	2	1	31	0	4	10	5	15	1
2	22	6	+	0	1	18	37	6	2	1	2	5	1
3	39	1	+	1	1	10	43	0	2	1	1	1	1
4	41	5	+	<1	<1	0	1	0	20	3	1	27	1
5	43	0	−	2	2	<1	42	3	3	1	3	1	1
6	13	0	−	9	9	7	30	0	22	3	5	2	1
7	11	14	+	0	16	1	1	0	42	12	1	2	1
8	18	0	+	2	4	1	3	0	34	18	5	15	1
9	15	0	−	0	0	0	0	0	50	31	2	2	1
10	36	28	+	0	1	7	17	0	3	3	<1	4	1
11	9	0	+	2	46	1	13	0	11	5	11	2	2
12	<1	<1	−	<1	32	7	7	0	17	19	3	4	2
13	<1	0	−	0	77	3	0	0	<1	16	2	1	2
14	8	3	+	0	12	16	46	0	5	0	7	3	3
15	4	<1	+	2	<1	13	46	0	13	8	3	10	3
16	3	0	−	0	3	64	12	0	4	4	7	3	3
17	12	0	+	10	3	59	13	0	<1	<1	2	0	3
18	13	0	−	0	8	44	21	0	6	2	2	4	3
19	1	0	−	0	2	59	29	0	4	1	2	1	3
20	3	0	−	1	4	55	25	0	4	2	3	3	3
21	1	2	+	7	0	<1	<1	0	80	1	2	6	4
22	5	4	+	15	0	<1	3	0	66	3	1	2	4
23	3	<1	−	10	1	0	0	0	74	2	5	4	4
24	4	<1	−	4	<1	1	1	1	16	69	2	4	5
25	2	0	−	0	0	11	7	0	19	58	2	1	5

*Three hundred cells counted.

[a]For descriptive purposes only, morphologic variations were grouped into different subtypes. Each subtype was grouped and arranged chronologically.

ing cases with a normal or absent karyotype (FISH not done; cases 19, 24, and 25), in one patient (case 25) both PML and RARα rearrangement was detected by Southern blot analysis, and in 2 patients (cases 19 and 24) only the RARα rearrangement was present. Cases 6 and 10 without t(15;17) on cytogenetics but with FISH positive also showed PML/RARα on RT-PCR, while case 22 was confirmed by Southern blot analysis (Table I).

Morphology

Bone marrow. Table II shows the bone-marrow differentials on 300 cells from 25 cases of APL where cytogenetic and/or molecular genetic evidence for the diagnosis of APL was present. Most cases of APL were composed, in various combinations, of a heterogeneous population of cells [30]. Seven major types of cells were observed, five of which are distinctive for APL: hypergranular cells, cells with multiple Auer rods [28], cells with dust-like granules often concentrated in one area of the cytoplasm [28], bilobed variant cells with no, fine, or few granules [1], and cells with basophilic cytoplasm and cytoplasmic projections [21]. The other two cells observed were the normal-appearing promyelocyte and the myeloblast. Infrequent residual cells of the more mature granulocytic series could be seen in some cases [41].

Based on the presence of these seven cells, five subtypes of APL were categorized: a hypergranular subtype and four microgranular/hypogranular subtypes. In one form of the hypergranular subtype, and in all microgranular sybtypes, a single cell predominated (⩾60%). (Table III). For descriptive purposes only, morphologic variations were grouped into different subtypes. Subtype 1 was characterized by a prominent hypergranular cell population. This subtype was frequently heterogeneous, and consisted of cells in various combinations, including numerous prominent hypergranular cells, cells with multiple Auer rods, cells with dust-like granules concentrated in one area of the cytoplasm, cells with basophilic cytoplasm and cytoplasmic projections (sometimes with granulation), some bilobed variant cells, and normal promyelocytes and myeloblasts (Fig. 1). In one form of the hypergranular subtype, hypergranular cells (often with bilobed, reniform nuclei) were predominant, together with cells with multiple Auer rods, corresponding to the description of the classical FAB M3 [28] (Fig. 2). Subtype 2 was the APL variant (FAB M3v) documented by the FAB Co-operative Group (1980) [1], in which many bilobed variant cells with few, fine, or no granules were observed (Fig. 3). The cells in the marrow could show

TABLE III. Morphologic Subtypes of APL

Hypergranular APL	Microgranular/hypogranular APL[a]
Heterogeneous population with prominent hypergranular cells (Fig. 1) or predominance of hypergranular cells together with cells with multiple Auer rods (Fig. 2) (FAB M3 [28]; subtype 1)	Predominance of bilobed variant cells (FAB M3v [1]; subtype 2) (Fig. 3)
	Predominance of cells with basophilic cytoplasm and cytoplasmic projections (subtype 3) [21] (Fig. 4)
	Predominance of differentiated promyelocytes with a few blast cells (subtype 4) (Fig. 5)
	Predominance of blast cells with early promyelocytes (subtype 5) (Fig. 6)

[a]The majority of cases had few hypergranular cells (<1–5%). APL with basophil [60–62], and eosinophilic [63] differentiation have been described.

more obvious granulation than seen in the peripheral blood [1,30]. Subtype 3 consisted mainly of basophilic cells with cytoplasmic projections (⩾60%), with or without cytoplasmic granules in the blue cytoplasm, the hypogranular form corresponding to the description of McKenna et al. in 1982 [21] (Fig. 4). In some of these cases the proportion of hypergranular cells was <5% (cases 15, 16, 19, and 20). The basophilic cell was also noted in most subtype 1 cases and was observed (>10%) in 16 of the 25 patients [21,30]. Subtype 4 (M2-like) consisted of 3 cases where the majority of cells (>60%) were normal-appearing, differentiated promyelocytes without evidence of folding and lobulation of the nucleus (Fig. 5). Few hypergranular cells were observed, and a diagnosis of FAB M2 could easily be made [42]. Subtype 5 (M1-like, 2 cases) consisted mainly of myeloblasts (~60%) with a small proportion of early promyelocytes and 2–4% hypergranular cells. It corresponded to FAB M1 [14], if the infrequent hypergranular cell was not recognized (Fig. 6).

A proportion of hypergranular cells was always observed in each subtype, ranging from the hypergranular subtype (subtype 1) with more frequent hypergranular cells, to cases where the hypergranular cell constituted <1–5% of the cell population, as could be seen in subtypes 2–5. In addition, in subtypes 1–4, cells with multiple Auer rods could be seen, but they were frequent in classical hypergranular FAB M3 (subtype 1), were much less frequent in subtype 2 and in some cases of subtype 3, and were not noted in subtype 5. Subtype 1 and most cases of subtype 2 were usually easily recognized, but subtypes 3–5 could be difficult to recognize if hypergranular cells were infrequent.

The 15;17 translocation was found in subtypes 1–5. In 2 cases (cases 19 and 24), t(15;17) was not proven, though the RARα gene rearrangement was detected. It is possible that in these cases the chromosome 17 segment was translocated to another chromosome as a cytogenetically undetectable variant rearrangement. In these latter 2 cases we were not able to formally exclude the possibility that the RAR rearrangement was involved in one of the rare translocations reported in APL, i.e., either (11;17) or (5;17) [43].

Peripheral blood. Table IV shows the peripheral blood differentials on 100 cells from the APL cases, together with the bone-marrow subtype. It will be noted that in hypergranular FAB M3 (subtype 1), leukopenia was present in 7 out of 10 patients. In one case (case 7), with leukocytosis, typical variant cells consistent with FAB M3v were present in the peripheral blood. The bilobed variant cell was observed in subtype 2 (FAB M3v) and in 5 of the 7 cases of subtype 3. In the latter type, variant cells with an intense blue cytoplasm often predominated. Because of the numbers of variant-type cells in the peripheral blood of subtype 3, many of these cases were classified as FAB M3v, despite the different bone-marrow morphology. In subtype 4, the 3 cases showed an increased number of promyelocytes together with myelocytes and metamyelocytes. In subtype 5, myeloblasts were the predominant cell. In both subtypes 4 and 5, APL would not have been the primary diagnosis considered.

Cytochemistry

Both the MPO and SBB stains were strongly positive in all cases except one (case 13), where MPO showed weak to moderate positivity. In 5 cases (cases 1, 3, 11, 22, and 24) where CAE was performed, all showed strong positivity and in 2 cases (cases 3 and 22), cells with multiple Auer rods were observed. NSE was performed on all 25 cases, and in 6 cases weak positivity of <10% of cells was noted.

Immunophenotyping

Immunophenotyping was performed on 24 patients. The panmyeloid marker CD33 (My9) was positive in 22 out of 22 cases where the test was performed. CD13 (My7) was positive in 19 out of 22 cases. A further 2 cases were positive with pooled CD33/CD13. In all cases, CD14 (My4) was negative, while HLA-DR was negative in all but one case. $CD33^+/CD13^+$, $HLA\text{-}DR^-$, and $CD14^-$ were found in 96% of cases (Fig. 7) [7,44,45]. Four cases had $CD34^+$ cells and were either subtype 2 or 3. CD2 was positive in 8 of 24 cases, including 2 out of 3 subtype 2 cases and 6 out of 7 subtype 3 cases. CD2 was thus found in cases with subtype 2 and 3 microgranular morphology in the bone marrow [3,46,47]; however, though the numbers were small, subtypes 4 and 5 were CD2-negative like hypergranular FAB M3. CD19 was positive in 2 cases and these cases were considered to be biphenotypic. Seven cases were tested with a CD56 antibody. Two cases (cases 23 and

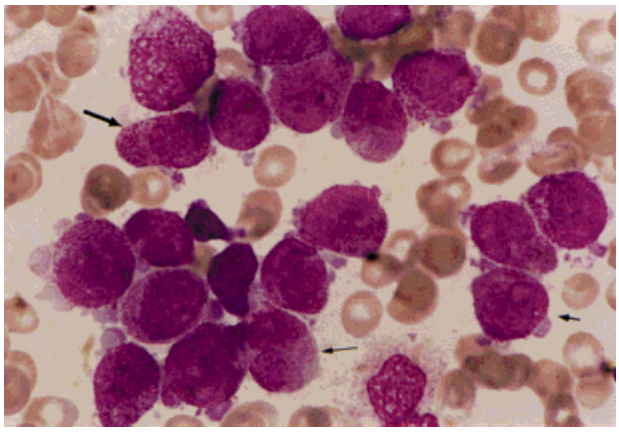

Fig. 1. Bone marrow of APL with heterogeneous population. FAB M3 with heterogeneous population in which hypergranular cells (➜), cells with blue cytoplasm and cytoplasmic projections (→), and a bilobed variant cell (⟶) may be seen. Occasional normal promyelocytes and myeloblasts are also seen (not shown). Subtype 1; case 6. PML-RARα on FISH and RT-PCR. Wright-Giemsa stain, ×1,000.

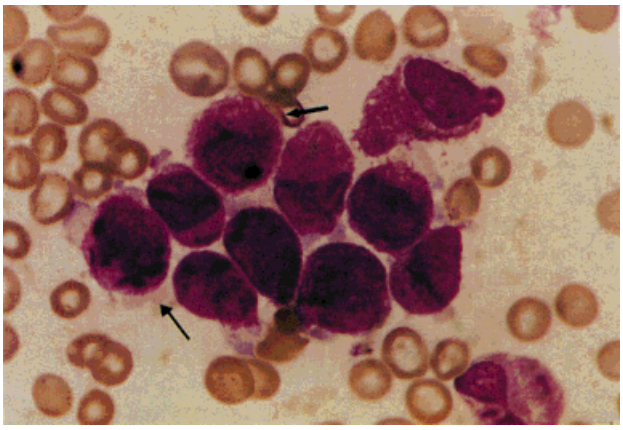

Fig. 2. FAB M3 in which hypergranular cells predominate (➜), and cell with multiple Auer bodies/rods is noted (➜). Subtype 1; case 10. PML/RARα on FISH & RT-PCR.

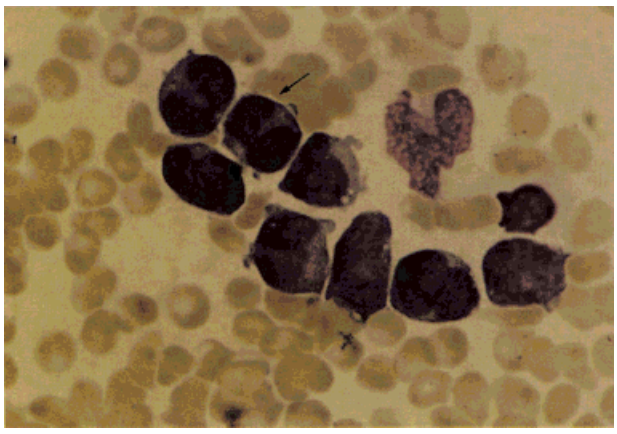

Fig. 3. Hypogranular cells with bilobed nuclei (→) typical of the FAB variant [1]. Subtype 2; case 13. t(15;17); PML/RARα on RT-PCR.

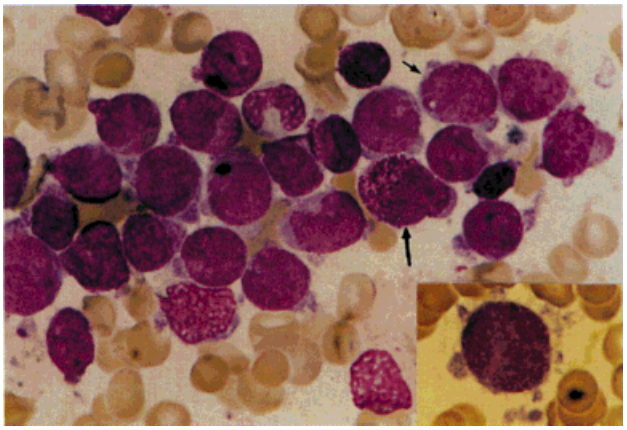

Fig. 4. Many basophilic cells, with cytoplasmic protrusions (→), are noted [21]. A single hypergranular cell is present (⟶). Insert: A typical cell with basophilic cytoplasm and cytoplasmic projections. Subtype 3; case 17. t(15;17); RARα.

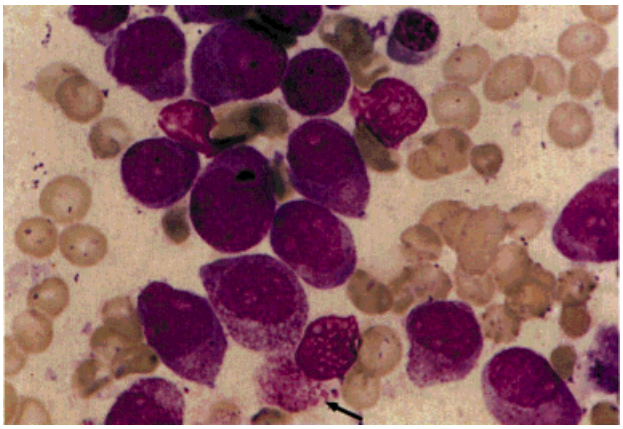

Fig. 5. M2-like APL in which normal promyelocytes predominate. Note disrupted hypergranular cell (→). Subtype 4; case 21. t(15;17); PML-RARα.

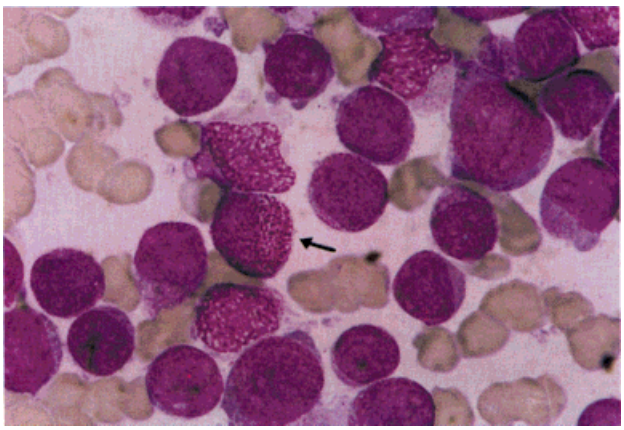

Fig. 6. M1-like APL. Note hypergranular cell (→). Subtype 5; case 25. PML-RARα.

Figs. 2–6. Bone marrow of APL cases. One type of cell (~60%) predominates. Wright-Giemsa stain, ×1,000.

TABLE IV. Peripheral Blood Cell Count (%) in 25 APL Cases*

Case no	Lkcs count $\times 10^9$/L	Hyper-granulation	Variant bilobed cell[a]	Fine dusting granules	Multiple Auer rods	Basophilic cytoplasmic projections without granules	Basophilic cytoplasmic projections with granules	Basophilic cytoplasmic projections with Auer rods	Regular blasts (M1)	Regular promyelocytes (M2)	Myelocytes to Neutrophils	Nucleated red-blood cells	Lympho-cytes	Subtype (bone marrow)
1	1.9	0	2	0	0	4	0	0	0	0	32	0	62	1
2	1.3		**										1	
3	2.0	7	7	1	0	12	9	0	4	4	17	3	36	1
4	0.5		,,	,,	,,	,,	,,	,,	,,					1
5	1.2		**											1
6	4.6	13	9	9	0	7	30	0	3	22	5	0	2	1
7	53.8	4	41	0	9	1	0	0	23	18	1	0	3	1[b]
8	1.6		**										1	
9	37.0	8	9	0	0	0	0	0	17	12	16	0	8	1
10	0.6		**										1	
11	13.5	9	46	2	6	2	13	0	5	11	10	0	2	2
12	35.0	1	67	0	0	0	24	0	5	4	3	0	2	2
13	19.5	0	68	0	0	1	0	0	6	0	4	0	21	2
14	8.0	0	52	5	0	1	1	0	3	2	23	2	11	3
15	1.0		**											3
16	2.1	0	0	0	0	0	0	0	12	5	65	3	15	3
17	23.9	2	77	0	0	1	0	0	5	0	6	0	9	3
18	16.7	2	19	0	0	3	1	0	20	9	11	0	5	3
19	8.6	0	41	0	0	2	0	0	4	0	33	4	15	3
20	7.1	0	40	0	1	1	0	0	9	10	8	6	25	3
21	11.2	0	6	0	1	0	0	0	9	28	51	0	5	4
22	5.8	0	2	0	0	3	0	0	10	28	27	5	25	4
23	13.1	0	7	0	1	0	0	0	0	55	29	0	8	4
24	5.1	0	0	0	0	0	0	0	51	2	11	1	35	5
25	169.0	0	0	0	0	0	0	0	90	1	8	0	1	5

*One humdred cells counted.
**Leukocyte (Lkc) count too low. Differential not done.
[a]Includes bilobed cell with basophilic cytoplasm.
[b]FAB M3V in peripheral blood.

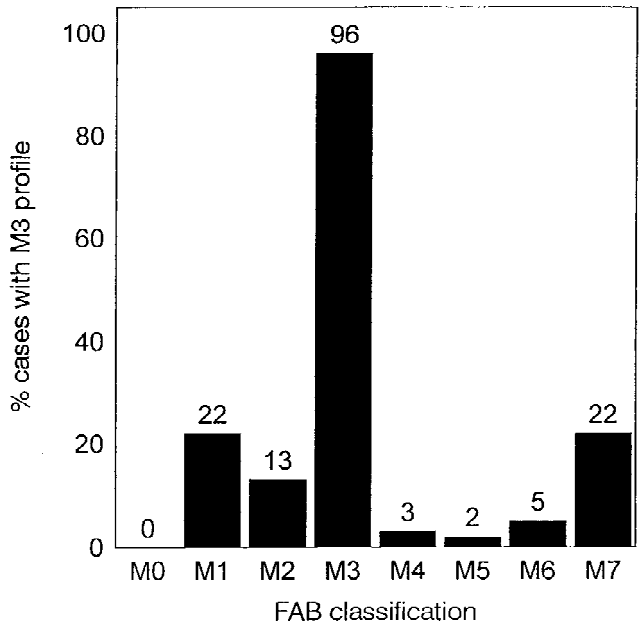

Fig. 7. Distribution of M3 immunophenotypic profile ($CD33^+/CD13^+$, $HLA\text{-}DR^-$, and $CD14^-$) in 548 AML cases according to the FAB classification. Includes M0 (0/14), M1 (31/144), M2 (16/127), M3 (23/24), M4 (4/131), M5 (1/61), M6 (1/20), and M7 (6/27) cases.

25) were positive ($CD33^+/CD13^+$, $HLA\text{-}DR^-$, $CD14^-$, and $CD56^+$) [48].

Disseminated Intravascular Coagulation

Twenty-one of 24 patients showed evidence of DIC which was not specific for any subtype (subtype 1, positive in 8 of 10 cases tested; subtype 2, positive in 3 of 3; subtype 3, positive in 6 of 7; subtype 4, positive in 2 of 3; and subtype 5, positive in 1 of 1 case tested).

DISCUSSION

When the FAB Group first described FAB M3 [28] they did not have the advantage of comparing their morphologic examination with other techniques, including cytogenetics [8], rearrangement of PML/RARα on Southern blot analysis [34], identification of the PML/RARα fusion transcripts by Northern blot anlysis [3] or RT-PCR [6,12,49], or identification of the PML/RARα protein by immunohistochemistry [25]. Subsequent to the identification of the 15;17 translocation [50], the FAB Group in 1980 recommended, when describing a variant form of APL (M3v), that all cases with t(15;17) in which the morphology was not that of typical M3 be assessed with the features of the M3 variant in mind [1]. In the revised criteria for the classification of AML published in 1985 [29], the FAB Group concluded that the morphologic interpretation for APL, which included both the classical hypergranular form and the typical variant, required no descriptive alteration. However, in 1982 McKenna et al. [21] had described 3 out of 39 cases of APL that included predominantly small hyperbasophilic cells with cytoplasmic projections in which an infrequent hypergranular cell was observed. In this paper we describe two additional subtypes, one consisting mainly of normal-appearing, differentiated promyelocytes and the other largely of myeloblasts.

APL frequently consists of a heterogeneous population of cells. As a result of the differentiation block occurring at the promyelocytic stage [3,19,51], seven major types of cells are seen. In most cases, various combinations and varieties of these cells, in which hypergranular cells are obvious, lead to the morphologic heterogeneity noted in APL (Fig. 1). On occasion, one of the cells predominates. This leads to five subtypes in which a single cell predominates. One is the classical FAB M3 variety with hypergranular cells and Auer rods (Fig. 2), and the other four are the hypogranular/microgranular subtypes including the typical FAB variant (Fig. 3), the subtype described by McKenna et al. [21] (Fig. 4), and the two additional subtypes, described here, one consisting mainly of regular-appearing promyelocytes (Fig. 5) and the other largely of myeloblasts (Fig. 6).

Our series has enabled us to expand on the bone-marrow description given by McKenna et al. [21]. Many of the cells with cytoplasmic protrusions were not hyperbasophilic, and varied from hyperbasophilia to cells with light blue cytoplasm. The nuclei, though often showing nuclear lobulation, could be oval or round. Furthermore, though many of the cases showed basophilic cells with few or no granules, some cases showed cells with variable granulation, and a few with hypergranulation. A number of these cells were also observed in the classical hypergranular form [21]. Rarely, typical basophilic cells with cytoplasmic projections showed no granules, but instead showed Auer rods in a clear blue cytoplasm.

The subtype described by McKenna et al. [21] (subtype 3) is probably a morphologic variant of the typical FAB M3v (subtype 2). Both have bilobed variant cells in the peripheral blood and CD2+. A proportion of the cells in both subtypes also shows CD34+, a primitive stem-cell marker. Some investigators have included this type under FAB M3v [2,52,53]. However, the bone marrow is different morphologically and consists predominantly of basophilic cells with cytoplasmic projections (Fig. 4) [21]. McKenna et al. [21] differentiated their cases from the typical FAB M3v variant and designated their cases under the heading ''microgranular variant,'' which included both their basophilic microgranular variant and the typical variant described by the FAB Group in 1980.

As recognized by Sultan et al. [54] and the FAB investigators [28], the key to the diagnosis of APL in the bone marrow is the recognition of the hypergranular cell.

Their number may be found as low as 1% or less of the cell population in some subtypes of APL (cases 12, 13, 19, and 21). The finding of cells with dust-like granules frequently concentrated in one area of the cytoplasm, of basophilic cells with cytoplasmic protrusions, and of cells resembling the typical variant cell with nuclear lobulation points to the diagnosis of APL in cases where the proportion of hypergranular cells is low. Thus it is possible in cases of subtypes 2–5, where the percentage of hypergranular cells is low, to recognize APL morphologically. Differentiation of subtype 4 with numerous differentiated promyelocytes from FAB M2 can be achieved by finding infrequent hypergranular promyelocytes, an occasional cell with multiple Auer rods, and strong MPO, SBB, and CAE staining. The most difficult subtype to recognize as APL is subtype 5 (M1-like), where hypergranular cells may be rare, and where cells with multiple Auer rods have not been observed. Recently Head et al. [14], when investigating 71 APL cases with t(15;17), found 3 cases resembling FAB M1, diagnosed at the primary institution and confirmed upon central review [55]. Two out of 2 cases tested showed PML/RARα by RT-PCR (Head, personal communication). This confirms our observation of M1-like APL cases. It should be noted that in these cases hypergranular cells were not specified [14].

It has since been suggested that all AMLs should have an RT-PCR performed for PML/RARα [56]. However, some hospital laboratories would not have the technical or fiscal resources to accomplish this. By better defining the morphology of APL, a select group of cases could undergo testing for PML/RARα using RT-PCR. This would allow rapid diagnosis of the most likely cases so that ATRA therapy could be given early to cases with PML/RARα.

The incidence of the various subtypes in our series may not be the true incidence found in the Hamilton region. Our cell-typing center is also a referral center for a large geographical area in Ontario, which leads to the referral of unusual cases from outside hospitals. Three out of the 7 cases of subtype 3 were referred from outside hospitals. The finding of a larger number of variant forms in our series may be the result of a selection bias or may be due to chance. We have observed M1-like APL (subtype 5) in 2 of 41 cases (4.9%). This is similar to the 3 of 71 cases (4.2%) noted by Head et al. in a large referral center [14, and personal communication].

We have found the immunophenotypic profile $CD33^+$/$CD13^+$, HLA-DR^-, and $CD14^-$ useful for indicating APL [7,44,45]. It was found in 96% of our APL cases. However, it is not specific for APL, being found in 11% of 524 non-M3 AML cases (Fig. 7). It differentiates M3v from monocytic FAB M5b, which is CD14+ and HLA-DR-positive. Biondi et al. [47] have shown that CD2 expession in APL is associated with microgranular morphology. This has been confirmed by our results where microgranular morphology (subtypes 2 and 3) was associated with $CD2^+$; however, the other microgranular forms (subtypes 4 and 5) have been CD2-negative, like hypergranular FAB M3 (subtype 1). We also noted $CD34^+$, a stem-cell marker, in 4 out of 10 subtype 2 and 3 cases. An immunologic profile for APL that includes CD9 positivity, using a Hybritech reagent (BA2), has been described by De Rossi et al. [57]. However, we found no specific relationship between $CD9^+$ and APL. $CD9^+$ has been found by us in all subtypes of AML (data not shown). We have not used the BA2 antibody used by De Rossi et al. [57], as it has not been available. Scott et al. [48] described a unique myeloid/natural killer cell (NK) leukemia that resembles M3v, but has an $CD33^+$/$CD13^+$, HLA-DR^-, $CD56^+$ immunophenotype, lacks t(15;17), and does not respond to ATRA therapy in vitro. Two out of 7 t(15;17) APL patients tested by us showed the same immunophenotype. Scott et al. [48] also noted 2 out of 50 t(15;17) APL patients with this phenotype. Finding M3v-like cases, on morphology, with the $CD33^+$/$CD13^+$, HLA-DR^-, $CD56^+$ immunophenotype will require exclusion from APL by cytogenetic or molecular genetic testing. We also found this phenotype in 5 of 66 non-M3 AML cases.

The early recognition of APL is important because of the indication for ATRA therapy in t(15;17) APL, and if not recognized and treated promptly, APL carries the risk of severe, and often fatal, coagulopathy [3]. Following the FAB morphologic criteria should allow the diagnosis of most cases of APL; however, the atypical forms of APL can be confused morphologically with other types of AML. Therefore, confirmation of the diagnosis has required cytogenetic analysis, gene rearrangement study, RT-PCR, or ultrastructural analysis [30,57]. The morphologic criteria for diagnosis, described here, can lead to rapid identification of most atypical cases so that appropirate APL therapy can be initiated early. With the recent use of RT-PCR to demonstrate PML/RARα transcripts [6,12,58] or immunohistochemistry to localize the PML/RARα protein [25], and in those cases where direct cytogenetic analysis is positive for t(15;17), it should be possible to rapidly confirm the morphological diagnosis.

The importance of recognizing patients with variant forms of APL and confirming them with other tests is underlined by the fact that in patients with equivocal morphologic characteristics and normal karyotypes, but typical molecular rearrangements of RARα, response to ATRA has occurred [23]. In regard to our patients, 3 out of 5 subtype 3 patients (basophilic cell predominance) remain in remission after ATRA therapy (cases 16, 17, and 20; 44, 31, and 19 months, respectively), along with 1 of 2 patients of subtype 4 (M2-like) (case 22; 24 months). One case (case 25) with subtype 5 morphology (M1-like) was placed on ATRA on admission because of

the morphological suspicion of APL (2% hypergranular cells among myeloblasts (Fig. 6); DIC). However, the patient died 3 days after initiation of therapy from sepsis and suspected pulmonary hemorrhage. Southern blot testing showed both RARα and PML rearrangement.

In conclusion, there are two main forms of APL: a common heterogeneous form in which hypergranular cells and multiple Auer rods are obvious, and a form where one cell type predominates. The latter consists of 5 subcategories: these include the classic FAB M3 with hypergranular cells with multiple Auer rods; the FAB variant with hypogranular bilobed cells [59]; a variant consisting of basophilic cells with cytoplasmic projections [21]; a form with numerous promyelocytes of normal appearance (M2-like); and a type consisting mainly of myeloblasts (M1-like). The last four are less common, with the last two being uncommon. Two microgranular forms, the FAB variant and the subtype with basophilic cells, are CD2+ [3,46,47]. A further variation of APL with basophil differentiation has been described [60–62]. Recently, a case of acute eosinophilic granulocytic leukemia with PML-RARα fusion gene expression has been reported. The patient achieved complete remission after ATRA therapy [63]. Recognition of the less common, atypical forms of t(15;17) APL is important because of the unique response of this disease to ATRA therapy.

ADDENDUM

We have recently seen an additional case of APL with undifferentiated morphology suggestive of FAB L2. Flow cytometry showed biphenotypic expression; CD2+, CD7+, CD13+, CD15+, CD34+, HLA-DR+, TdT+, CD33−, CD14−, CD5−, CD19−. Peroxidase staining showed 8% blast positivity and the occasional Auer rod and the diagnosis of FABM1 was made. After receiving therapy for AML, the patient developed DIC. RT-PCR showed PML-RARα transcripts (short form) indicative of APL (a variant form with CD2+). It is recommended that cases of AML with DIC should have RT-PCR undertaken for PML-RARα.

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